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Imbalance of Inflammatory Cytokine Response Induced by Acute Chloroform Toxicity In Vivo

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A B S T R A C T

Chloroform (CH) is an environmental pollutant that induces serious health effects. This experimental study was designed to investigate the serum cytokine response induced by acute exposure to chloroform. Eighteen adult male laboratory rats were separated into three groups of eight rats each. The first control (CON) group included animals without any treatment, while the rest of the two groups were administrated trichloromethane at a dose of 477 mg/kg , orally then sacrificed after 1 day (CH-1) and 7day (CH-7) post-exposure. The results indicated that acute exposure resulted in immune disrupting through a significant increase in pro-inflammatory cytokine levels compared to unexposed control rats. In contrast, chloroform intoxication caused a remarkable decrease in the anti-inflammatory (IL-10) level in the serum of euthanized rats on 1st day after exposure. In conclusion, chloroform intoxicated caused alteration in serum inflammatory cytokine response.

INTRODUCTION

Chloroform ($CHCl_3$) is a colorless, sweet-smelling, volatile organic compound [1] that was historically used as an anesthetic [2] but is now primarily utilized in laboratory settings and the manufacturing of various industrial products [3]. Due to its toxicity and associated health risks [4], chloroform is classified as a hazardous substance and is subject to strict regulatory controls to ensure safe handling and disposal [5]. Chloroform exposure is potentially severe in a variety of settings, including occupational, manufacturing, and laboratory settings [6], as well as through intentional ingestion or inhalation for recreational purposes [7] or suicide attempts [8]. Acute and chronic exposure to chloroform has been linked to heightened risks of liver and kidney diseases and other health complications [9,10]. Due to its lipid solubility, chloroform can easily traverse lipid membranes, affecting highly vascularized organs such as the brain, liver, heart, and kidneys [11,12]. It can be generated during chlorination of drinking water and is a common contaminant in drinking water [13,14].In laboratory animal models, a strong association between toxicity and elevated serum levels of certain inflammatory mediators has been demonstrated [15-17]. However, there is an urgent need to evaluate the effect of hazardous environmental toxins on serum levels of immune cytokine responses. Therefore, this study was designed to determine the serum cytokine response in rats acutely exposed to chloroform.

METHODS AND MATERIALS

Chemicals

The materials used in this study included ketamine at a concentration of 10% (100 mg) and xylazine at 20 mg/ml, sourced from Alfasan (Woerden, Netherlands) and KELA (Hoogstraten, Belgium). Choloroform was sourced from (CAS N:67-66-3, Dr MOJALLALI Industrial Chemical Complex Co, Iran).

Animals and groupings

For this experiment, 18 mature male Wistar rats from the laboratory animal centers were consumed and housed in specially designed cages that provided optimal conditions, including a 12-hour light/dark cycle, temperatures maintained between 20 and 25 ºC, and humidity levels ranging from 50 to 60 %. Throughout the study, all animals were fed a standard pellet diet and had continuous access to water.Before the study began, the animals underwent a one-week acclimatization period to help them adjust to their new environment, ensuring their well-being and the reliability of the study outcomes. All experiments were conducted under control and accordance to the Laboratory Animals Experiments Ethical Committee (LAEEC), Urmia University. The rats were divided into 3 control and experimental groups, each consisting of 6 rats. The control group received no treatment (CON). The tow experimental group received trichloromethane (one dose at 477mg/kg, orally). The chloroform-received rats were euthanized on day 1 (CH-1) and 7 after chloroform administration (CH-7). Following experiment termination, the rats were euthanized with ketamine and xylazine and blood samples from cardiac punctures were taken, put in designated tubes, processed to extract the serum, and then kept cold at– 40 ° C until analysis.

Assessing levels of inflammatory cytokines

The concentrations of IL-6, IL-1β, IL-10, IL-8, TNF-α, and TGF-β in serum samples were evaluated using a standardized ELISA protocol. Serum samples were collected, processed, and diluted based on industry recommendation. Standards were created through serial dilution according to the kit instructions.

Statistical analyses

The SPSS (version 25) software was applied to process all results, and M± SD was the output. To determine the differences between the studied groups, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc analysis interpretation. The significance level was set at $p < 0.05$. GraphPad Prism was utilized to design the figures.

RESULTS

Chloroform increased pro-inflammatory cytokines level in serum

Observations revealed an important increasing - depending time in serum level of IL-6 on 1st and 7th days compared to control rats ($p<0.0001$). Furthermore, the rats administered chloroform exhibited a notable increase in serum IL-8 and IL-1β levels compared to control. This increase in IL-1β levels was time-dependent. However, there was no significant variance between 1st and 7th days post-chloroform groups related to IL-8 level ($p >0.05$). Additionally, serum levels of TNF-α and TGF-β were compared between studied groups. The results indicated that chloroform significantly elevated both TNF-α and TGF-β serum levels versus to control animal. The increase in TGF-β was time-dependent, whereas no significant change was observed in TNF-α levels following chloroform administration in one and seven day (Figure 1-5).

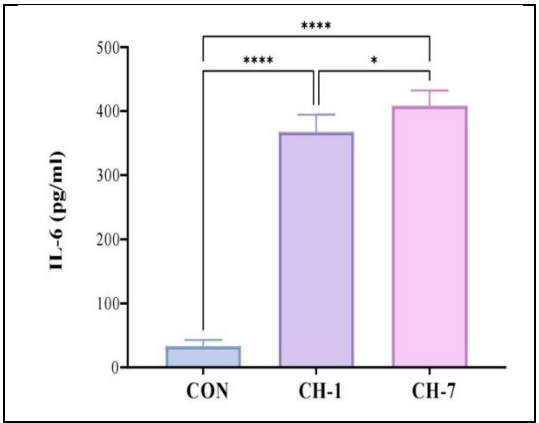

Figure 1: Toxic effect of chloroform on serological levels of IL-6 in studied groups. *: significant at ($p < 0.05$); **** ($p<0.0001$).

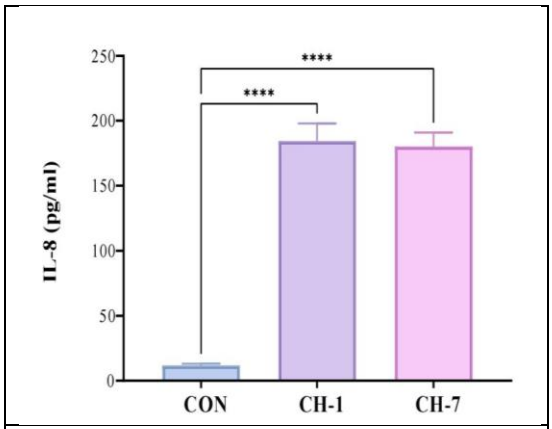

Figure 2: Toxic effect of chloroform on serological levels of IL-8 in studied groups. ****; significant at ($p<0.0001$).

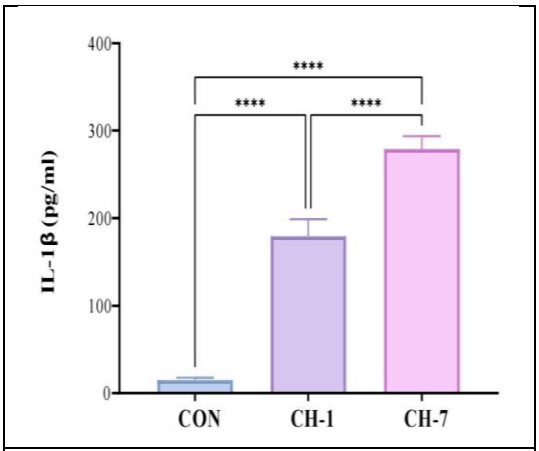

Figure 3: Toxic effect of chloroform on serological levels of IL-1β in studied groups. ****; significant at ($p<0.0001$).

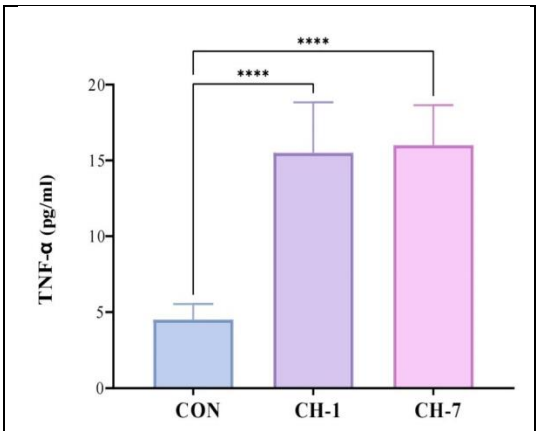

Figure 4: Toxic effect of chloroform on serological levels of TNF-α in studied groups. ****; significant at ($p<0.0001$).

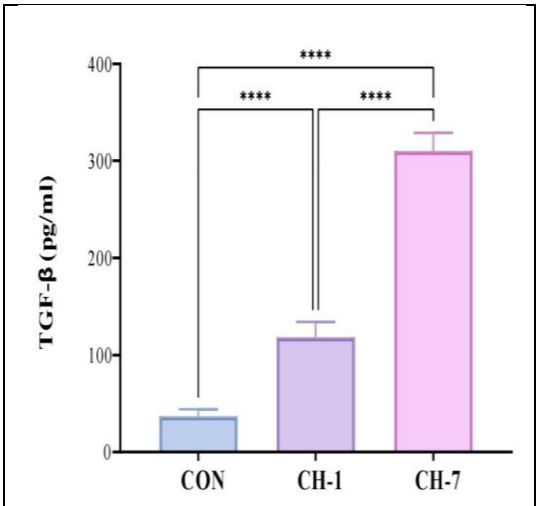

Figure 5: Toxic effect of chloroform on serological levels of TGF-β in studied groups. ****; significant at ($p<0.0001$).

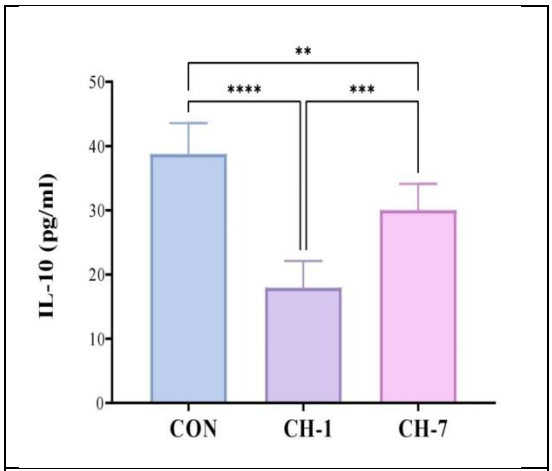

Figure 6: Effect of chloroform on serum levels of IL-10 in studied groups.**: significant at ($p < 0.01$), *** ($p < 0.001$); **** ($p<0.0001$).

Chloroform decreased anti-inflammatory cytokines level in serum

Observations demonstrated a significant ($p<0.0001$) decrease in the serum level of IL-10 on day one after chloroform administration. However, it was increased on day seven versus day one post-chloroform administration ($p < 0.001$). Although the serum level of IL-10 was increased on day seven post-chloroform administration, it was significantly ($p < 0.01$) lowered than control group (Figure 6).

DISCUSSION

Acute exposure to chloroform can lead to central nervous system depression, while chronic exposure may result in hepatotoxicity [18]. Chloroform metabolism in the liver and kidneys produces toxic metabolites that contribute to hepatotoxicity and nephrotoxicity, and its inhibition of key enzymes in the electron transport chain disrupts ATP producing, causing energy depletion and cellular damage [19,20]. When chloroform is metabolized in the liver, primarily by cytochrome P450 enzymes, it is converted into reactive metabolites, including phosgene [21]. Phosgene is highly toxic and can deplete glutathione, an important antioxidant, thereby indirectly damaging hepatocytes and initiating inflammatory reactions that can affect the liver and other parts of the body [22]. Consequently, the effects of chloroform can be monitored in the serum. To our knowledge, no scientific articles have reported the acute effects of chloroform on serum levels of inflammatory cytokines. Therefore, our study was performed to impact the acute effect of chloroform on serum levels of pro-inflammatory and anti-inflammatory cytokines in rats exposed to chloroform on days one and seven. Our findings demonstrated that chloroform exposure significantly increased serological levels of IL-6, IL-8, and TNF-α versus control animals. This increase occurred in a time-dependent manner following chloroform exposure. These results suggest that acute exposure to chloroform may initiate immuno-toxic pathways that can be developed in long-time exposure. Moreover, an increased level of IL-8 in the serum is indicative of a higher likelihood of neutrophil infiltration [23,24]. Also, we found that chloroform significantly increased IL-1β and TGF-β levels in the serum. Indeed, IL-1β has a critical role in promoting inflammation and injury by lowering the expression of adhesion molecules and other cytokines, thereby enhancing the recruitment and activation of inflammatory cells in the serum [25,26]. Excessive TGF-β signaling promotes the activation of HSCs and leads to increased extracellular matrix deposition, ultimately causing liver fibrosis and cirrhosis [27].On other hand, IL-10 represents an anti-inflammatory cytokine which regulates the immune responses and protects several tissues against excessive inflammatory reactions [28]. It has the ability to inhibit the production of pro-inflammatory cytokines [29], and by reducing the overexpression of inflammatory cytokines, it contributes to protecting the liver from damage caused by excessive immune responses [30].Besides, it reduces the activation of immune cells, which are sources of inflammatory mediators. This helps to limit inflammation and injury [31]. Moreover, the IL-10 promotes cells survival through reducing apoptotic signaling under stress conditions [32]. Our findings indicated that the serum level of IL-10 was significantly reduced in rats administered chloroform. However, IL-10 levels increased on day seven post-administration compared to day one. These changes suggest that during the acute phase, IL-10 levels markedly decrease, whereas in the sub-acute phase, they increase (though not to baseline levels) as a protective mechanism to mitigate severe inflammatory responses such as apoptosis [33]. Although further investigations are required to fully elucidate the signaling pathways activated by chloroform, including those related to apoptosis, it can be concluded that chloroform significantly induces pro-inflammatory reactions, as evidenced by elevated levels of pro-inflammatory cytokines, while suppressing the release of anti-inflammatory cytokines like IL-10. This imbalance may play a pivotal role in metabolic processes.

CONCLUSION

The results provided novel insights into the acute toxic effect of chloroform exposure on inflammatory biomarkers in rats. The data reveal an important increase in pro-inflammatory cytokines in response to chloroform administration. These cytokines are crucial mediators of inflammation and damage, suggesting that chloroform exposure induces a potent inflammatory response. The study also highlights a marked decrease in the anti-inflammatory cytokine IL-10, which further exacerbates the inflammatory state and suggests a compromised regulatory inflammation response.

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